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Microbial Inhibition by an Isolate of *Pediococcus* from Cucumber Brines[1]

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We reported earlier that *Pediococcus cerevisiae* FBB-61 inhibited *Lactobacillus plantarum* FBB-67 in mixed species inoculation used for the fermentation of brined cucumbers. Herein, 16 isolates of the *Pediococcus* genus from various sources were tested for inhibitory activity against *L. plantarum* and other microorganisms by a seeded-agar screening technique. Only two of the 16 isolates gave consistent and distinctive zones of inhibition, and both were isolated from fermenting cucumber brines on separate occasions. These two isolates did not inhibit each other but did inhibit the other 14 *Pediococcus* isolates in addition to *L. plantarum*. They also inhibited several other gram-positive bacteria, but not four species each of gram-negative bacteria and yeasts tested. Inoculation of cucumber juice broth with *P. cerevisiae* FBB-61 and *L. plantarum* WSO resulted in a drastic reduction in the plate count of *L. plantarum* WSO during day 1, but counts increased rapidly thereafter. Consequently, acid production by *L. plantarum* WSO was delayed. Noninhibitory isolates of *Pediococcus* had no appreciable effect on growth and acid production by *L. plantarum* WSO.

Mixed species inoculation of brined cucumbers with *Pediococcus cerevisiae* and *Lactobacillus plantarum* has the potential advantage of an early, rapid initial growth and moderate acid production by the former species and a higher final acidity resulting from the lower pH tolerance of the latter (5). The presence of *P. cerevisiae* FBB-61, however, delayed the growth of *L. plantarum* FBB-67 in such mixed species inoculations (5); delays up to 10 days have been noted (J. L. Etchells, T. A. Bell, and R. N. Costilow, U.S. Patent 3,403,032, 1968).

The objective of the present work was to compare inhibitory properties of *Pediococcus* cultures, from various sources, against lactic acid bacteria associated with the fermentation of brined cucumbers and against certain other bacteria and yeasts.

Cucumber juice broth (CJB; 6) diluted to 65% by volume with water and containing 5% NaCl was used for single and mixed species fermentations. Differential plate counts of *L. plantarum* WSO and the inhibitory *P. cerevisiae* FBB-61 culture were made in a basal medium consisting of 4% Trypticase, 0.5% gelatin, 2% agar, and 0.02% bromocresol green (5) with 2% sugar, either sucrose or glucose. Sucrose was autoclaved separately and added aseptically to the sterilized basal medium. *L. plantarum* counts were obtained from the sucrose medium, since the inhibitory *P. cerevisiae* isolates did not form visible colonies in this medium. Total counts were obtained from the glucose medium, and *Pediococcus* counts were calculated from the difference in counts between the two media. Plates were incubated at 30 C. With this procedure, not all cultures could be differentiated from *L. plantarum*, because some noninhibitory *Pediococcus* cultures also formed colonies in the sucrose agar.

Pediococcus cultures were screened, by an agar spot test, for ability to produce substances inhibitory to other microorganisms. The surface of solidified Trypticase soy agar (BBL; 0.2% glucose added), in petri dishes 100 mm in diameter, was spot inoculated by pipette with a drop of broth culture of the *Pediococcus* culture (effector) to be tested for inhibitor production. A maximum of four cultures, spaced approximately 3 cm apart, was spotted per dish. The inoculated agar dishes were incubated for 1 day or longer at 30 C and then overlaid with 5 ml of

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Trypticase soy soft agar (0.5% agar in Trypticase soy broth, BBL), which had been seeded with 0.1 ml of the bacteria or yeast culture to be tested for sensitivity. A clear zone of 0.5 mm or greater extending laterally from the border of the *Pediococcus* colonies after incubation for 1 day at 30 C was recorded as positive inhibition. Cultures for spot inoculation of agar surfaces and for adding to the soft agar overlay were grown in Trypticase soy broth for 16 to 20 h at 30 C; they were added undiluted as indicated above.

By the agar overlay technique, 16 cultures of the *Pediococcus* genus were tested for inhibitory activity against other bacteria and yeasts. Two of the 16 cultures consistently produced inhibition zones of 1 mm or greater (*P. cerevisiae* FBB-61 and L-7230) when tested against *L. plantarum*. Both cultures were isolated from fermenting cucumber brines, one in Michigan and the other in North Carolina. Although these two cultures did not inhibit each other, they did inhibit the other pediococci tested, including two also isolated from cucumber brines and certain other gram-positive bacteria (Table 1). Zones of inhibition remained upon extended incubation, even after the *Pediococcus* colony was killed with chloroform vapor prior to overlaying with soft agar seeded with the test microorganisms. None of the gram-negative bacteria or yeasts tested was inhibited. All of the lactic acid bacteria listed in Table 1 were tested as the effector organism, but only *P. cerevisiae* FBB-61 and L-7230 consistently gave zones of inhibition. Occasionally *P. cerevisiae* ATCC 8081 gave a small zone (<0.5 mm) when tested against *L. plantarum* WSO.

TABLE 1. *Inhibition of microorganisms by P. cerevisiae FBB-61 grown on agar surface*[a]

Cultures inhibited	Cultures not inhibited
P. cerevisiae (FBB-39, L-728, B-1325,[b] B-1326,[b] E-66,[c] ATCC 8081[d])	*P. cerevisiae* L-7230
P. pentosaceus (183-IW, P-20, P-2)[d]	*Lactobacillus brevis* 50
P. acidilactici (135, 146, SP-4, SP-5, SP-7)[d]	*Salmonella typhimurium*
L. plantarum (WSO, 442, 68, 15)	*Pseudomonas aeruginosa*
Leuconostoc mesenteroides 42	*Escherichia coli* (K-12, B)
Micrococcus luteus	*Aerobacter aerogenes* (1, 2)
Streptococcus faecalis	Yeasts[b]
Stahylococcus aureus ATCC 10537	*Pichia membranaefaciens* NRRL Y-1627
Bacillus cereus T	*Debaryomyces membranaefaciens* NRRL Y-1455
	Saccharomyces rosei NRRL Y-1567
	Candida krusei NRRL Y-105

[a] Cultures were from collections in the Department of Microbiology, Michigan State University, or from the Food Fermentation Laboratory, Raleigh, N.C., unless specified otherwise. It is recognized that some or all of the *P. cerevisiae* cultures used herein may eventually be shown to be more closely identified with one or more other species according to the recent taxonomic classification of the genus *Pediococcus* (1).

[b] These cultures were from the Northern Regional Research Laboratory (NRRL), U. S. Department of Agriculture, Peoria, Ill.

[c] This culture was from J. R. Stamer, Cornell University.

[d] These cultures were from J. B. Evans, Department of Microbiology, North Carolina State University.

CJB was inoculated with 10^6 cells of either *L. plantarum* WSO or *P. cerevisiae* per ml, singly or in combination. When added in combination, *P. cerevisiae* FBB-61 inhibited acid production by *L. plantarum* WSO (Fig. 1). Acid production by the mixed species fermentation paralleled that for the pure culture fermentation by *P. cerevisiae* FBB-61 for about the first 10 days. Then, the acidity in the mixed species fermentation increased, but it never reached the final level attained by *L. plantarum* WSO alone (Fig. 1). *P. cerevisiae* L-7230 also delayed acid production by *L. plantarum* WSO. When either of two other *P. cerevisiae* cultures (FBB-39 and L-728) was added in combination with *L. plantarum* WSO, the acid production curves showed no evidence of inhibition and followed closely the curve of *L. plantarum* alone (Fig. 1). Further, neither of these pediococci was shown to be inhibitory by the agar screening test.

The presence of a mixture of *L. plantarum* WSO and the inhibitory *P. cerevisiae* FBB-61 in CJB, at about 3×10^6 cells of each per ml, reduced plate counts of *L. plantarum* to less than 1 per ml during the first 24 h of incuba-

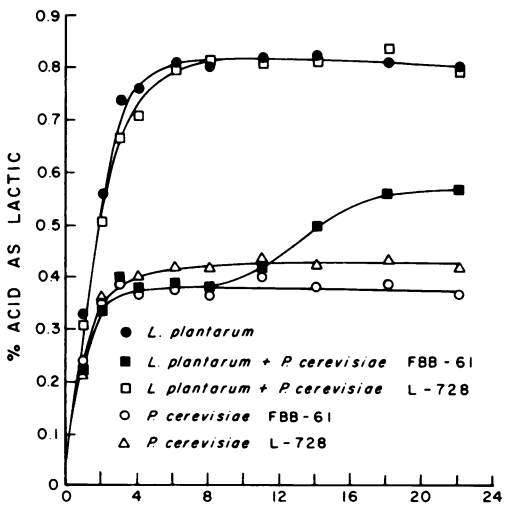

FIG. 1. *Acid production by L. plantarum WSO, P. cerevisiae isolates, and combinations of the two species in CJB containing 5% NaCl. Temperature, 30 C.*

tion. *L. plantarum* counts increased after the 2-day inhibitory period to a maximum of about 3×10^7 cells/ml after 8 days. In contrast, *L. plantarum* alone reached 6×10^8 cells/ml after 1 to 2 days. These findings are similar to those reported earlier in pure culture fermentations of brined cucumbers (5), except for the precipitous drop in *L. plantarum* counts during the first 24 h, which was not noted earlier (5) because samples were not plated between 0 and 24 h. This period apparently is very critical for expression of the inhibitory action. We are uncertain whether the failure of *L. plantarum* WSO to form colonies when plated during this period was due to death of these cells or to bacteriostatic action of the pediococci also present in the plated agar.

Hydrogen peroxide production of certain lactobacilli has been reported to inhibit other bacteria (2, 8). The addition of filter-sterilized catalase (Calbiochem) at a level of 300 IU/ml to Trypticase soy agar used in screening tests and to CJB in mixed culture inoculations did not prevent inhibition of *L. plantarum* WSO by *P. cerevisiae* FBB-61 and demonstrated that H_2O_2 did not cause the inhibition.

P. cerevisiae FBB-61 and L-7230 were catalase positive when grown on the surface of Trypticase soy agar and were gram-positive cocci appearing in single, double, and tetrad cell arrangements, as viewed microscopically in wet mount. They were not gas formers, produced acid in CJB containing 5% NaCl, and attained a final pH of 3.4; but, in pure culture-fermented cucumbers, the final pH is usually about 3.7 (5; Etchells et al., U.S. Patent 3,403,032, 1968).

P. cerevisiae has been reported by Haines and Harmon to inhibit *Staphylococcus aureus* (7). Those workers did not report the inhibition of other lactic acid bacteria by pediococci, nor that only certain strains of pediococci have the unusual antimicrobial properties noted herein. It is now clear for the first time that certain isolates of *Pediococcus* from fermenting cucumber brines may inhibit other lactic acid bacteria important to the fermentation of brined cucumbers and other vegetables, as well as certain other bacteria. Thus, care must be taken in the selection of the *Pediococcus* cultures to be used in mixed species inoculations for the controlled fermentation of cucumbers (3), olives, and other vegetables (4) brined in bulk. Furthermore, the inhibitory nature of certain pediococci may explain the initial occurrence of this lactic species, prior to the development of *L. plantarum* in natural fermentations of brined cucumbers (Etchells et al., U.S. Patent 3,403,032,1968) and Spanish-type green olives (4).

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